REMARKS

The foregoing amendments and these remarks are filed in response to the Non-Final Office Action dated March 2, 2010 (the "Office Action"), along with a Request for Extension of Time. Authorization is hereby given to charge fees for a two month extension of time, as well as charge any underpayment or credit any overpayment to Deposit Account No. 50-0951.

At the time of the Office Action, claims 159-181 were pending. In the Office Action, objections were raised regarding claim 171. Claims 160, 163, 165, 167 and 176 were rejected to under 35 U.S.C. §112, second paragraph. Claims 159-181 were rejected under 35 U.S.C. §103. All of the rejections and responses thereto are set out fully below.

The new claims are fully supported by the specification and recite alternate subject matter. Support for new claim 182 may be found, for example, on page 37 lines 4–30 original claim 26 of the PCT Application.

Support for new claim 183 may be found, for example, on page 29, lines 27, and page 30 lines 6, and triamplification in page 39, lines 14 and claims 25, 58 and 59 and Fig 6 of the PCT application. The only difference being three oligonucleotides have been used instead of four and two mentioned. However, fourth labeled oligonucleotide can be used but use of labeled oligonucleotides has been restricted to three labeled oligonucleotide primers because of examiner's restriction. This claim involves target amplification using ligase and polymerase. This amplification is triamplification and the last option off line is ligation PCR amplification. Ligase chain reaction as a predisclosed nucleic acid amplification method wherein four oligonucleotides and a ligase enzyme only are used no polymerase is used and has been referred in background of this application as one of the amplification process in page 4, line 27 of the PCT specification. Triamplification as a nucleic acid amplification process has been mentioned in page 39, line 14, and original claim 23 of the PCT application which can also be achieved by the method of the present invention.

Support for new claim 184 may be found, for example, original claim 61 of the PCT application and strand displacement amplification on pages 39, line 13 of the PCT application

Support for new claim 185 may be found, for example, on page 29, lines 27 – page 30 lines 6, and original claims 58 and 59 of the PCT application. It is restriction of PCT claim 14 and addition of ligase enzyme. In this claim only two labeled oligonucleotide primers have been used. This claim involves target amplification using ligase and polymerase. Ligase chain reaction as a predisclosed nucleic acid amplification method wherein four oligonucleotides and a ligase enzyme only are used and has been referred in background of this application as one of the amplification process in lines 27 page 4 of the PCT application.

I. Claim Objections

Claim 171 was objected to for minor informalities. Appropriate amendments have been made herein and withdrawal of the objections is respectfully requested.

II. 35 U.S.C. § 112 Rejections

Claims 160, 163, 165, 167 and 176 were rejected to under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants believe appropriate amendments have been made herein. Withdrawal of the rejection is respectfully requested.

III. Rejection under 35 U.S.C. §103(a)

Claims 159-168, 171-176, and 178-180 were rejected under 35 U.S.C. §103(a), as being unpatentable over U.S. Patent No. 5,866,336 to Nazarenko et al. ("Nazarenko"), Solinas et al., "Duplex Scorpion primers in SNP analysis and FRET applications, "Nucleic Acids Research, October 15, 2001, Vol. 29, NO. 20, pp. 1-9, Oct. 15, 2001 ("Solinas") and U.S. Patent No. 6,117,635) Nazarenko et al. ("Nazarenko (2000)"). Claims 169, 170, and 177 are rejected under 35 U.S.C. §103(a) as being unpatentable over Nazarenko, Solinas, and Nazarenko (2000) as applied to claim 159 above, and further in view of U.S. Patent No. U.S. 6,210,897 to Andersson et al. ("Andersson") and PCT Publication No. WO 1993/17126 to Chetverin et al. ("Chetverin"). Claims 159-168, 171-176, and 178-180 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nazarenko, PCT Publication No. WO 1998/113524 to Sato et al. ("Sato") and

Nazarenko (2000). Claims 169, 170, and 177 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nazarenko, Sato, and Nazarenko (2000) as applied to claim 159 above, and further in view of Andersson. and Chetverin. Lastly, claim 181 is rejected under 35 U.S.C. 103(a) as being unpatentable over (1) Nazarenko, Solinas, and Nazarenko (2000) as applied to claim 159 above, or (2) Nazarenko, Sato. and Nazarenko (2000) as applied to claim 159 above; and further in view of Webb et al., Accession No. M60048, 1993 ("Webb") and Buck et al., "Design Strategies and Performance of Custom DNA Sequencing Primers," BioTechniques 27:528-536, September 1999 ("Buck"). Claim 159 is believed to be patentable over the cited art.

Regarding use of linear oligonucleotides, Nazarenko discloses a donor moiety located on a first oligonucleotide primer and an acceptor located on a second, complementary oligonucleotide, the 3' end of which is not free to be extended by a polymerase. This clearly indicates that amongst a pair of labeled oligonucleotides, one is a labeled oligonucleotide primer and the other is an oligonucleotide being a complementary oligonucleotide to the labeled oligonucleotide primer and therefore is not a primer but is designed to hybridize to the labeled oligonucleotide primer to form a single duplex.

The pair of oligonucleotides separately labeled with donor and acceptor FRET moieties of the Applicants present invention are the oligonucleotide primers, each set to prime at the two opposite strands of the target sequence in the nucleic acid amplification reaction, and do not form a duplex amongst itself. In contrast to the labeled duplex primer of Nazarenko, in which only one of the labeled oligonucleotides of the duplex is a primer and the other labeled complementary oligonucleotide is not a primer, and instead is a blocking oligonucleotide; in the duplex primer of claim 161 in the present application, both the labeled oligonucleotides of the duplex primer are primers.

Nazarenko uses linear labeled oligonucleotides, as either both donor and acceptor FRET labels which are on one linear primer, or the donor and acceptor FRET labels are on a primer and a complementary blocking oligonucleotide, in which a signal is generated from incorporation of doubly labeled FRET primer or labeled primer labeled blocking oligonucleotide duplex into

amplification product and measuring signal after separating donor and acceptor moieties of the unincorporated primer and blocking oligonucleotide.

The main objective of Nazarenko is incorporation of a detectably labeled (with molecular energy transfer label) primer duplex in the amplification product and detection of such amplification product and direct detection. Wang mentions the use of a donor labeled reverse primer and acceptor labeled energy sink oligonucleotide complementary to the reverse primer, that form a primer duplex which is equivalent to the labeled blocking oligonucleotide and labeled reverse primer duplex of triamplification of Nazarenko. Nazarenko does not use any probe and also incorporates the labeled primer and labeled blocking oligonucleotide duplex in the amplification product and generate signal from the same. Thus, Applicants confirm Nazarenko did not target at specificity determination by probe type hybridization of the blocking oligonucleotide and focused on the issue of primer dimer formation. Nazarenko also teaches the use of a duplex primer as discussed above, an oligonucleotide primer is a hairpin primer labeled with a donor-quencher FRET pair that forms a hairpin structure in which FRET occurs when it is not incorporated in the amplification product and when the primer gets incorporated into the amplification product it becomes linearized whereby quenching is eliminated and fluorescence signal from the donor is detected.

Further, Nazarenko 2000 teaches labeled linear oligonucleotide(s). In one of the embodiments, the donor-acceptor FRET pair is located on the same single stranded oligonucleotide primer and in another embodiment the donor is located on a first oligonucleotide and the acceptor is located on the second oligonucleotide wherein one of the two FRET labeled oligonucleotides is a primer for triamplification and the other FRET labeled oligonucleotide is a blocker for triamplification, where the blocker is a linear molecule with blocked 3' end and complementary to the labeled primer. Thus, there is no suggestion that detection with high specificity occurs at the torget nucleic acid by just two FRET labeled forward and reverse primers. To the contrary, blocking of the 3' end of the blocking oligonucleotide incorporating biotin at 3' end to avoid unwanted non-specific products (including primer dimer) suggests

formation of primer dimer by Nazarenko's way of use of labeled oligonucleotide. Further, Nazarenko did not have any solution to primer dimer and other non-specific product formation.

The use of the reverse primer-blocker duplex of Nazarenko is not at all effective in preventing mis-priming between the amplification primers, as the 3' end of the primer protrudes out of the duplex and the single stranded 3' end of the forward primer is accessible to the 3' end of the reverse primer-blocker duplex.

Use of blocking oligonucleotide in the triamplification process as taught by Nazarenko may reduce the non-specific priming or non-specific amplification to some extent, specifically when the blocking oligonucleotide is sufficiently complementary to the primer, thereby not allowing priming of the primer to the non-specific priming sequence, but cannot rule out nor help in the reduction of primer dimer formation, thus rendering the method of Nazarenko non-specific. Further, sufficiently complementary blocking oligonucleotide will also inhibit priming of the primer to the priming sequence due to competition between priming sequence and blocking oligonucleotide for the primer resulting in inhibition of the amplification process and any inhibition of amplification results in primer dimer formation. Accordingly, Nazarenko is not very effective and leads to considerable loss of sensitivity. In Nazarenko (column 25 lines 64–66), it is mentioned that the blocker is substantially complementary to at least a portion of the reverse primer. If the blocker is substantially complementary to a portion of the reverse primer, then protection against non-specific priming will not be good. Again, if it is substantially complementary to reverse primer then protection against non-specific priming will be good but as a consequence, specific priming by the primer will be inhibited. The primer dimer formation cannot be eliminated much by using reverse primer–blocker duplex, as there will be amplification inhibition due to such use of duplex and amplification inhibition results in more primer dimer. Even with hairpin primer, there will be inhibition in amplification due to sluggish opening of hairpin. In this context, it should be remembered that the method of Nazarenko does not use any probe.

Further, if one considers the teachings of Nazarenko (column 15, lines 45-53) as discussed above, wherein the oligonucleotide primer is a hairpin primer labeled with a donor-

quencher FRET pair, it is obvious to a person skilled in the art that there would be competition between two strands of the hairpin energy transfer primer with target sequence when the primer sequence is part of the stem of the hairpin, and the strand of the duplex stem complementary to the primer sequence will have more affinity towards the primer sequence as the primer sequence and the complementary oligonucleotide are part of the same molecule (intermolecular interactions are more favored than intermolecular interactions) wherein opening of the hairpin will be sluggish, which inhibits target amplification.

For the forgoing reasons, Nazarenko approaches to directly detecting or measuring a product of nucleic acid amplification reaction (as mentioned in Column 7 lines 2 - 12) would lead to reduced sensitivity and specificity. Thus, the disclosure of Nazarenko is not adapted to inhibit signal from primer dimmer and eliminate non-specific signal generation.

In Solinas, the two FRET moiety labeled oligonucleotides of the duplex scorpion are a probe sequence and a quencher oligonucleotide, complementary to the probe sequence, which are labeled at the 5' end of the probe with ROX and Methyl Red and at the 3' end of the quencher oligonucleotide respectively, where the 3' end of the probe sequence is linked to the 5' end of the primer through a stopper. These two oligonucleotides cannot be extended by polymerase because of their blocked 3' end. Thus, the two oligonucleotides of Solinas are oligonucleotides of a duplex oligonucleotide probe and not a primer dimer, which is different from duplex probe, and the oligonucleotides cannot be and is definitely not a primer. Accordingly, formation of primer dimer between these two oligonucleotides is ruled out. Primer dimer can only form between two primers, the forward and reverse primers in the nucleic acid amplification reaction wherein in this case the primer dimer cannot generate a signal. Further, inhibition of primer dimer or non-specific amplification product does not arise as the primer sequence of the scorpion can form primer dimer with other primer, use of probe sequence supports the aforesaid whereby probe is used to generate signal from specific amplification product. Moreover, this method is based on use of a probe, and use of a probe is known to inhibit amplification reaction resulting in loss of sensitivity and primer dimer formation. Further, use of dual labeled (FAM & ROX) probe impairs probe hybridization because of lowering of

melting temperature of probe hybridization from such dual labeling of probe and the probe can easily be displaced by the advancing polymerase both resulting in loss of signal. Moreover, the polymerase while extending other primer hybridizing to the target strand to which the labeled probe hybridizes can remove the ROX fluorophore at 5' end of the hybridized probe by its 5' – 3' exonuclease activity thus resulting in loss of signal. In addition, due to lowering of probe melting temperature primer annealing and extension needs to be carried at lower temperature to facilitate probe hybridization. Further, Applicants invention is not at all targeted at the accomplishment of the primer dimer. Rather, it rules out the formation of primer dimer by labeling both the forward and the reverse primer with MET/ FRET moiety at least 2 bases away form their 3' ends unlike the labeled blocking oligonucleotide and labeled reverse primer of Nazarenko so that two labeled primers can be used to monitor or detect a target sequence with higher signal to noise ratio and higher specificity. Such labeling dispositions and only labeling of both the forward and the reverse primers to reach to the sensitive method of the Applicants present invention thereby inhibiting the formation of primer dimer is never hinted at by either Solinas or Nazarenko either taken alone, or in combination, as they fail to involve the use of two MET/ FRET labelled primers with their 3' ends free in their entire specification.

The selective use of two labeled primers and disposing the labels at least 2 bases away from the 3' ends of each of the primers provide for the qualifying detection method according to the Applicants invention that is free of primer dimer formation in the Applicants novel and inventive detection method that could never have been an obvious extension from the prior art since it is only by way of the advancement studied under the present invention reflecting from the disclosure in examples 7 and 8 page 52 lines 15 – page 54 lines 19 that such surprising and unexpectedly special signal generation and quantification of the target sequence could be achieved. None of the prior art have either dealt with or even hinted at such possible advancement in the detection method involving the combination of the labeled primers with the selective disposition of the labels at least two bases away from their 3' ends as qualified under the present claim 159. Prior art methods have used single labeled primer, labeled primer and labeled probe combination for nucleic acid target detection, but nobody has looked into or

thought of using two labeled primers for nucleic acid target detection because of the problems associated with use of such labeled primers. Before the present invention, primer dimer formation was a significant problem. The Applicants present disclosure is directed to solving prior art limitations of non-specific amplification by presenting a simple and rapid method that attends to unwanted formation of the primer dimer and non-specific amplification product involving the not known use of labeled forward and a labeled reverse primers selectively disposed with MET/FRET labels at least two bases away from their 3' ends to arrest the formation of the unwanted primer dimmer. Thus, since the method of the invention involves use of MET/FRET labeled forward primer and MET/FRET labeled reverse primer selectively disposed at least two bases away from 3' ends wherein the said labeling on both the forward and the reverse primer and the said specific position at which it is labeled acts in combination to achieve the objects of the invention and accordingly to disassociate one of the steps of the invention of oligonucleotides at least 2 bases away from 3' end is not desirable since the method need to be traversed in totality which none of the prior art does or even hints at.

The invention teaches use of two MET/FRET labeled oligonucleotide forward and reverse primers labeled at least two bases away from their 3' ends that can prime a polymerase dependent nucleic acid amplification reaction to amplify a target sequence without formation of non-specific amplification product and primer dimer whereby the signal is generated through interaction of the MET/ FRET moiety on each of the two labeled primers only when the target sequence is correctly amplified in the amplification product. It thus discloses an unexpected observation that simultaneous labeling of both forward and reverse amplification primers allows target detection without formation of non-specific amplification product and primer dimer. Further placement of labels two to four bases away from their 3' end help in avoiding primer dimmer formation.

Placement of labels at a distance less than 2 base away from 3' end result in increased primer dimer formation is disclosed wherein Nazarenko teaches placement of label 2 and 1 base away from 3' end of primer. Nazarenko uses one labeled primer and the labeled primer is a duplex (hairpin primer and reverse primer-blocking oligonucleotide) primer, duplex primer can

help in reducing non-specific amplification product to some extent but it does not help in completely eliminating reducing primer dimer formation as discussed above, and our experience with duplex primer has not been good. Disclosure of Solinas is attachment of fluorophore and quencher labeled quenched duplex probe to one of the two unlabeled amplification primers for efficient and fast intramolecular (rather than less efficient intermolecular) probe hybridization and not based on MET/ FRET moiety labeled forward and reverse primers of the Applicants present disclosure.

Regarding claim 159, Applicants respectfully submit that Nazarenko teaches incorporation of a duplex (formed of a labeled primer (reverse) and a labeled blocking oligonucleotide, wherein the said labeled blocking oligonucleotide is not a primer and is sufficiently complementary to the labeled primer) into an amplification product in triamplification, whereas the labeled primer and blocking oligonucleotide are labeled separately with a donor moiety and an acceptor moiety and the other amplification primer (forward) is used unlabeled. In contrast, Applicants method employ a labeled forward primer and a labeled reverse primer and not a duplex primer as used by Nazarenko, where the labeled primers are labeled separately with a donor and an acceptor moiety and get incorporated into the amplification product.

Further, Nazarenko uses additional 5' and 3' modifications of the blocking oligonucleotide and a 2-OMe modification of the labeled primer, an extra oligonucleotide in addition to two primers and a ligase, whereas the method of Applicant uses only two labeled primers in which no additional modification of the primers, no additional blocking oligonucleotide, and no ligase is required in their method of detection and/ or quantification of correct nucleic acid sequence. Further, the disadvantages associated in the use of labeled blocking oligonucleotide and labeled primer duplex in triamplification reaction of Nazarenko has been described in previous sections.

Regarding column 15 lines 23 – 26 of Nazarenko, Nazarenko mentions use of linear primer labeled with donor and acceptor in one oligonucleotide in PCR (Column 29 lines 58 – Column 30 lines 3), use of labeled reverse primer and labeled blocking oligonucleotide and

unlabeled forward primer of triamplification as discussed above (Column 30 lines 25 – lines 44) and in addition use of dual labeled (labeled with a donor and an acceptor) forward primer in combination with a dual labeled (labeled with a donor and an acceptor) reverse primer and unlabeled blocking oligonucleotide, or unlabeled forward primer and dual labeled (labeled with a donor and an acceptor) reverse primer and unlabeled blocking oligonucleotide in triamplification (Column 30 lines 44 – 59). If both primers of triamplification are used dual labeled there will be increase in signal due to individual contribution from each primer separately. In this case, the dual labeled primer is an independent entity for signal generation. A signal is generated after separating donor and acceptor moieties from an unincorporated dual labeled primer with 3'-5' exonuclease treatment only, where there is interaction between labeled moieties on any of the dual labeled primers, there is no interaction between label moieties on the forward primer and label moieties on reverse primer, and there is no energy transfer between donor or acceptor on forward and acceptor or donor on reverse primer. This result is achieved because there is a sizable gap between the forward and reverse primers. The amplification reaction is a gap version triamplification (Column 30 line 25) and the positioning of the donor and acceptor moieties do not allow such interaction.

In claim 167 the target to be amplified is a synthetic or artificial target that contains a target sequence and an additional non-target sequence. Claim 167 has been amended to make this point more clear.

The Office Action asserts that Nazarenko (2000) teaches second and third oligonucleotide primers with different labels. The Applicants respectfully submit that in case of the Applicants present disclosure, the third oligonucleotide is a primer in general and when it is not a primer it is used to just quench the donor or acceptor on two primers and not for signal generation unlike that of Nazarenko. The third oligonucleotide of claims 1–4 and column 20 lines 21–66 in Nazarenko is a third nucleotide sequence of the hairpin primer. The construct of the hairpin primer has been described in column 20 lines 21–66 where the hairpin primer is a composite oligonucleotide made of four different nucleotide sequences joined to each other. The joining of these four nucleotide sequences has been clearly mentioned by Nazarenko as a first

nucleotide sequence, a second nucleotide sequence, a third nucleotide sequence, and a fourth nucleotide sequence in 5' to 3' order (i.e., the first nucleotide sequence is at extreme 5' end and the fourth nucleotide sequence is at extreme 3' end,) where the fourth nucleotide sequence is the primer sequence (column 20 lines 47–52 Nazarenko). All four nucleotide sequences together make a single oligonucleotide, and each nucleotide sequence is a part of one hairpin oligonucleotide. Further, the third nucleotide sequence is having the second nucleotide sequence joined at it's 5' end and the fourth nucleotide sequence joined at it's 3' end. There is no existence of a third oligonucleotide sequence. Thus, the third nucleotide sequence cannot be and is not a third oligonucleotide.

Turning now to Sato, the Office Action asserts that Sato teaches the method of detection and/or quantification of a target nucleic acid sequence and/or a nucleic acid amplification reaction using nucleic acid amplification. However, Applicants submit that the distinguishing aspects of the Applicants present disclosure teach away from the method of Sato. Sato teaches the detection of a nucleotide sequence in sample in which there is no amplification of target nucleic acid. For detection of the target sequence Sato uses pair of labeled probes that hybridizes sequentially next to each other to a single stranded target nucleic acid thus forming a duplex of target strand and two adjacent probes, and the probes are labeled with a donor or an acceptor moiety and the labels are placed internally as well as at 5' and /or 3' end. On hybridization of the two linear probes to specific target sequence, donor and acceptor moieties are separated by 4–20 bp resulting in a FRET signal. In paragraph 0034, this distance of 4–20 bases between donor and acceptor when two labeled probes hybridize to target strand is mentioned. In paragraph 0041, different modifications of the probes have been mentioned. In table 1, different donor and acceptor fluorophore combinations or pairs have been given in column 1, single or double stranded structures between donor and acceptor moieties and the nucleotide spacing between donor and acceptors of probes in different probe hybridization formats have been given in columns 2 and 3 of the table. Paragraph 0096 gives the probe sequences with 5' and internal amino modification of probes for attachment to donor or acceptor. Paragraph 117 mentions Cy5 dye labeled probes. In Fig 1B, both the probes are labeled internally and are placed next to each

other, in Fig 1D both the probes are labeled internally and are placed a few bases away. In Figure 5, fluorescent emission on hybridization of a Biodipy P donor labeled probe and a CY5 acceptor labeled probe to a target sequence has been given. The method of Sato is based on hybridization of two single stranded probes labeled separately with donor or acceptor to a target sequence and they hybridize to one strand of a target sequence. The method does not involve any target amplification. The distinguishing aspects are firstly the method of Sato is a probe-based method, wherein the labeled oligonucleotides used are not primers as no target amplification is involved. Further, the labeled probes of Sato can never be used in nucleic acid amplification based target detection as the 3' ends of the probes are free and not blocked for polymerase extension.

Nauck has disclosed use of labeled (donor or acceptor) probe pair in nucleic acid amplification based target detection, Nauck has blocked 3' ends of the probes against extension by polymerase. Thus, the method of Sato is not relevant to the method of Applicant as the method of applicant, which is based on target amplification and specific detection of target sequence without using any probe. Moreover, there is serious primer dimer formation and non-specific amplification product formation problem issues with linear probes with free 3' ends and the Applicants submit that the use of linear probes by Sato does not give any clue for use of labeled probes as labeled primers.

Independent claim 159 is thus patentable over the cited prior art for the foregoing reasons. The dependent claims are also believed allowable because of their dependence upon an allowable base claim, and because of the further features recited.

IV. Conclusion

Applicant has made every effort to present claims that distinguish over the prior art. All claims are believed to be in condition for allowance. Nevertheless, Applicant invites the Examiner to call the undersigned if it is believed that a telephonic interview would expedite the prosecution of the application to an allowance. In view of the foregoing remarks, Applicant respectfully requests reconsideration and prompt allowance of the pending claims.

Respectfully submitted,

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